ATTACHMENT II – PROTOCOL

ECOLAB
Study Identification Number 1200052

REGULATED PESTICIDE EFFICACY STUDY PROTOCOL

STUDY TITLE: Aqualogic Germicidal Spray Hospital Disinfection Efficacy

EPA REG. NO.: 1677-

STUDY IDENTIFICATION NUMBER: 1200052

PROPOSED STUDY INITIATION/COMPLETION DATES

Initiation May 18, 2012

Completion July 18, 2012

DESCRIPTION OF STUDY OBJECTIVE

Aqualogic (EPA Registration No. 1677-) will be tested according to Ecolab Microbiological Services SOP Method MS010-20; *Germicidal Spray Products as Disinfectants* to determine hospital disinfection efficacy against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella enterica* ATCC 10708 after a 5 minute exposure time at room temperature (15-30°C) when diluted to 0.0660% free available chlorine in sterile Milli-Q or laboratory purified water per the Confidential Statement of Formula (CSF). The actual dilutions which will be performed for the test substance use-solutions will be determined subsequent to the chemical quality verification to deliver the required level of active ingredient, and documented in the raw data. The test substance will be challenged by the addition of 5% fetal bovine serum to the test systems. The test substance will be applied to the carriers at a distance of 6-8 inches for 3 sprays. AOAC Method 961.02, Chapter 6 Disinfectants, 2009 was the test method utilized in making the disinfectant claim.

TEST SUBSTANCE IDENTIFICATION

Test Substance Name: Aqualogic

Batch Identification:

1. **051512DT**
2.
3. **050112DT***

*Indicates the batch that will be tested as the 60 day aged batch.

One of the test substance batches will be amended into the study. All three batches will be used to determine the use-solution chemical quality verification analysis.

An aliquot of each test substance batch will be retained in the retention cabinet at ECOLAB Schuman Campus until the quality of the formula no longer affords evaluation. Test substance not dispersed for retention, chemical quality verification or efficacy testing will be stored in ECOLAB Microbiological Services laboratory until disposed.

QUALITY ASSURANCE UNIT MONITORING

The protocol, chemical quality verification in-life inspection, chemical quality verification in-life data audit, pesticide efficacy in-life and final report are proposed to be inspected by the ECOLAB Quality Assurance Unit (QAU) in accordance with their current Standard Operating Procedures. The following proposed ECOLAB QA inspections are for planning purposes only and may change. ECOLAB QA inspections that are performed, along with their dates and auditors, will be included in the study final report. Changes in ECOLAB QA inspections from those proposed below will not require revision of this protocol.

A. Proposed QAU Monitoring

Protocol Audit
Chemical Quality Verification In-Life Inspection
Chemical Quality Verification Data Audit
Pesticide Efficacy In-Life Inspection
Final Report Audit

CHEMICAL QUALITY VERIFICATION

A. Proposed Experimental Initiation/Termination Dates

Experimental Initiation Date: May 24, 2012

Experimental Termination Date: July 1, 2012

B. Method

Chemical analysis will be performed on each test substance batch to determine the concentration of the active ingredient. Chemical analysis will also be performed on the test substance use-solution. The use-solution preparation will be documented in the raw data.

The test substance is a ready-to-use product that will be diluted at or below the lower certified limit for the active ingredient. The following calculation will used to determine the amount of test substance in a 1,500 g use-solution diluted to 660 ppm (or 0.0660%) free available chlorine:

ppm at LCL = (% LCL/100) (specific gravity) 10^6 =
ppm at LCL = (0.0660%/100) X (0.999) X 10^6 = 659 ppm

Amount of Test Substance needed to be at or below the LCL =

$$\frac{\text{ppm at LCL} \times 100 \times \text{g amount of use-solution to be made}}{(\%\ \text{active})\ 10^6} = \text{grams of Test Substance}$$

In order to prepare the use-solution using weight to weight measurements, the specific gravity was incorporated into the calculations resulting in 659 ppm (or 0.0659%) free available chlorine as the lower certified limit.

The chemical quality verification will be performed by the Analytical Lab using the method listed below. The method has been deemed acceptable by the Analytical Lab and the study sponsor to ensure proper characterization of the test substance. Statistical treatment of test results may be inherent to the method. Additional volumes and dilutions may be necessary to determine the chemistry of the use-solution samples.

QATM-007; Available Chlorine
Available chlorine content is determined by reduction of the chlorine to chloride by iodide ion. The iodine liberated by this reaction is then determined by titration with sodium thiosulfate, either manually or potentiometrically with an automatic titrator.

The most current QATM will be used during the course of this study for the chemical and physical analysis.

C. Interpretation of Results

The concentration of the active ingredient in the test substance batches will be judged acceptable for pesticide efficacy testing if within the range specified by the Confidential Statement of Formula (CSF) upper and lower certified limits as seen in the table below.

Active Ingredient	CSF Lower Certified Limit	CSF Upper Certified Limit
Free Available Chlorine*	0.0660%	0.1030%

*The equivalent weight of NaOCL (sodium hypochlorite) to the equivalent weight of Cl_2 (Chlorine) is 37.2/35.5 = 1.05. Dividing the sodium hypochlorite concentration by the ratio of the equivalent weight of sodium hypochlorite to the equivalent weight of chlorine results in the free available chlorine concentration.

The concentration of the active ingredients in the test substance diluted to the lower certified limit (test substance use-solution) will be judged acceptable for pesticide efficacy testing if within the acceptance limit of 0.0594 – 0.0726% available chlorine.

After diluting the ready-to-use test substance to the lower certified limit of 0.0660% free available chlorine, the nominal concentration of the active ingredient is <1.0%. Therefore, the Calculated Lower Acceptance Limit and Calculated Upper Acceptance Limit for available chlorine will be expanded to accommodate method variability and suitable rationale. The expanded ranges are based on 40 CFR § 158.350 (Certified Limits) and was calculated as shown below.

Calculated Lower Acceptance Limit for available chlorine
= [0.0660% - (0.0660 x 0.1)] = 0.0594%
Calculated Upper Acceptance Limit for available chlorine
= [0.0660% + (0.0660 x 0.1)] = 0.0726%

The chemical quality verification raw data will be reported in the final report of this study.

PESTICIDE EFFICACY TESTING

A. <u>Proposed</u> Experimental Start/Termination Dates

Experimental Start	May 24, 2012
Experimental Termination	July 1, 2012

ECOLAB
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B. Methods

Pesticide efficacy data will be generated by the Microbiology Lab using the most current methods listed below. See the specific methods in the Protocol Appendix.

Method Number	Method Name
MS002-15	*Organic/Inorganic Soil Addition for One-Step Cleaner Disinfectant or Sanitizer Claims*
MS010-20*	*Germicidal Spray Products as Disinfectants*
MS088-17	*Test Substance Use-Solution Preparation for Analysis*

*MS010 will be followed with the following exception:
The test system culture will be uniformly spread over the carrier to within approximately 1/8 inch of the edges.

Test Method Requirement and Test System Justification

The following apply when determining the effectiveness of a hospital disinfectant; 60 carriers are required on each of three samples, representing different batches one of which is greater than 60 days old. The required organisms were *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella enterica* ATCC 10708. The AOAC Germicdal Spray Products Test for the above stated organisms are recommended based on the U.S. EPA Office of Chemical Safety and Pollution Prevention Product Performance Guidelines 810.2200 Disinfectants for Use on Hard Surfaces –Efficacy Data Recommendations March 12, 2012. Also, U.S. EPA Office of Chemical Safety and Pollution Prevention Product Performance Guidelines 810.2000 General considerations for Public Health Uses of Antimicrobial Agents March 12, 2012 applies to this study.

Test Method Justification

Ecolab Microbiological Services SOP MS010-20; *Germicidal Spray Products as Disinfectants* will be the test method utilized in this study.

Test Systems and Identification

The test systems which will be utilized for this procedure *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella enterica* ATCC 10708. Identification will be performed by observing the colony morphology.

Organic Soil Load

5% Fetal Bovine Serum

Test Substance Diluent

Sterile Milli-Q water or Sterile laboratory purified water

Test Substance Concentration

Antimicrobial efficacy testing will be performed with **Aqualogic** diluted to at or below the lower limit of 660 ppm.

Active Ingredient	CSF Lower Certified Limit	CSF Upper Certified Limit
Available Chlorine	0.0660%	0.1030%

The dilution procedure is based on results of the Chemical Quality Verification study. To achieve dilution of the ready-to-use test substance to at or below the lower certified limit of available chlorine, the test substance batches will be prepared based on the available chlorine results and documented in the raw data. The following calculation will be used to determine the dilution procedure for each test substance batch to result in the lower certified limit of available chlorine.

ppm at LCL = (% LCL/100) (specific gravity) 10^6 =
ppm at LCL = (0.0660%/100) X (0.999) X 10^6 = 659 ppm

Amount of Test Substance needed to be at or below the LCL =
$\frac{\text{ppm at LCL X 100 X g of use-solution to be made}}{\text{(\% active) } 10^6}$ = grams of Test Substance

Test Surface

Microscope slides, non-corrosive, 25 x 25 mm (1 x 1")

Exposure Time/Temperature

The test systems will be exposed to the test substance for 5 minutes at room temperature (15-30°C).

Spray Distance and Number of Trigger Sprays

The test substance will be applied by spraying the carriers at a distance of 6-8 inches for 3 sprays.

Neutralizer/Subculture Medium

Letheen Broth with 0.5% Sodium Thiosulfate

Plating Medium

Tryptone Glucose Extract Agar

Incubation Time/Temperature

Tubes and plates will be incubated for 48 ± 4 hours at 35 ± 2°C.

Test Controls

The following controls will be incorporated with the test procedure for each test system:

a. Average Volume/Weight Delivered per Carrier
b. Carrier Enumeration
c. Positive Control
d. Negative Control
e. Diluent Sterility
f. Fetal Bovine Serum Sterility
g. Neutralization Confirmation
h. Test System Purity

Details on each of the above controls can be found in Ecolab SOP MS010-20 located in Protocol Appendix.

Verification of Test System in Positive Subculture Tubes

All positive tubes from the test will be subcultured to Tryptic Soy Agar and Mannitol Salt Agar (for *S. aureus*), Pseudomonas Isolation Agar (for *P. aeruginosa*) and MacConkey Agar (for *S. enterica*). The colony morphology observed will be compared to the typical colony morphology of the test system for verification of the test system in positive subculture tubes.

Interpretation of Test Results

The performance standard for a disinfectant requires the product to kill the test organism on at least 59 out of 60 carriers to provide significance at the 95 % confidence level.

DATA RETENTION

Following completion of the study, an exact copy of the final report and the original raw data and protocol will be transferred to ECOLAB Archives at the ECOLAB Schuman Campus in Eagan, MN or an approved off-site location. All records that would be required to reconstruct the study and demonstrate adherence to the protocol will be maintained for the life of the commercial product plus four years.

TEST SUBSTANCE RETENTION

An aliquot of each batch of test substance will be retained in the retention cabinet at the ECOLAB Schuman Campus in Eagan, Minnesota until the quality of the formula no longer affords evaluation.

GOOD LABORATORY PRACTICES

This study will be conducted according to Good Laboratory Practices, as stated in 40 CFR Part 160. If it becomes necessary to make changes in the approved protocol, the revisions and reasons for change will be documented, reported to the sponsor and will become part of the permanent file for that study. The sponsor will be notified as soon as it is practical whenever an event occurs that could have an effect on the validity of the study.

- **Name and Address of Sponsor**

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  Eagan, MN 55121-1560

- **Name and Address of Testing Facility**

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- **Name of Study Director**

  Lisa Hellickson
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Sponsor

05/18/2012
Date

Study Director

18 May 2012
Date

PROTOCOL APPENDIX

Microbiological Services (MS) Methods:

MS002-15	*Organic/Inorganic Soil Addition for One-Step Cleaner Disinfectant or Sanitizer Claims*	Pages 1-3
MS010-20	*Germicidal Spray Products as Disinfectants*	Pages 1-9
MS088-17	*Test Substance Use-Solution Preparation For Analysis*	Pages 1-6

Standard Operating Procedure

Ecolab Controlled Document

ECOLAB
MICROBIOLOGICAL SERVICES

TITLE: **Organic/Inorganic Soil Addition for One-Step Cleaner Disinfectant or Sanitizer Claims**

NUMBER: **MS002-15**

EFFECTIVE: **04/01/11**

1.0 PURPOSE

The addition of organic soil to a test procedure is necessary to allow for the one-step cleaner-disinfectant or cleaner-sanitizer claim.

2.0 SCOPE

2.1 An antimicrobial product that bears the label claim of a one-step cleaner-disinfectant or cleaner-sanitizer, or one intended to be effective in the presence of organic soil, must be tested for efficacy by the appropriate method(s) which have been modified to include a representative soil such as 5% fetal bovine serum (e.g. blood serum).

3.0 STORAGE & HANDLING INSTRUCTIONS FOR FETAL BOVINE SERUM

3.1 Fetal bovine serum is to be stored at ≤ -10°C and used prior to the expiration date on the bottle label.

3.2 Fetal bovine serum may be thawed and dispensed into vials. Thawed fetal bovine serum may be stored at 2 - 8°C for up to two months from the date thawed.

3.3 Document the following information on Form 3070:

- Fetal Bovine Serum manufacturer
- Lot number
- Bottle identification
- Bottle expiration date
- Thawed/dispensed date
- New expiration date
- Initial & Date

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TITLE: **Organic/Inorganic Soil Addition for One-Step Cleaner Disinfectant or Sanitizer Claims**

NUMBER: **MS002-15**

4.0 TEST REQUIREMENTS

4.1 A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces with an organic soil load involves contamination of the appropriate carrier surface with each test microorganism culture containing 5% (v/v) fetal bovine serum (e.g. 19 mL test system and 1 mL fetal bovine serum) prior to the specified carrier-drying step in the method.

Note: The organic soil should be added to the test system suspension prior to inoculation of the test surface or test substance.

4.2 A 5% level of organic soil is considered appropriate for simulating lightly or moderately soiled surfaces. When the surface to be treated is heavily soiled, a cleaning step must be recommended prior to application of the antimicrobial. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level according to a specific label claim.

4.3 If an antimicrobial product has a soap scum claim, an appropriate method of simulation would be to add a 0.005% concentration of sodium stearate. A 1:20 dilution of a 0.1% solution of sodium stearate is made into the culture inoculum for a final concentration of 0.005%

4.4 An alternate method of introducing the organic soil where the antimicrobial agent is not tested against a dry inanimate surface involves adding 5% organic soil to the use-solution of a product prior to inoculation with the test system (e.g. 4.75 mL use-solution + 0.25 mL fetal bovine serum before adding 0.5 mL of the required level {5 x 10^6/mL} of conidia).

5.0 REGULATORY EFFICACY REPORTS

5.1 The level and type of organic soil must be stated in the protocol, raw data and the final efficacy report. The method of incorporation of organic soil must also be stated (e.g. 5% fetal bovine serum added to the test system suspension for the inoculation of the test surface or test substance).

5.2 The Certificate of Analysis for the fetal bovine serum shall be included in the study file.

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TITLE: Organic/Inorganic Soil Addition for One-Step Cleaner Disinfectant or Sanitizer Claims

NUMBER: MS002-15

6.0 RECORD MAINTENANCE

6.1 Records will be stored in the Test System binder located in Microbiological Services. Records from the current and previous year will be kept in the Microbiological Services Equipment Maintenance binder. All earlier records will be archived in the first quarter of the current year. For example, records from 2010 will be archived by March of 2012. Records will be transferred to ecolab Archives at the Ecolab Schuman Campus in Eagan, MN or to an approved off-site location.

7.0 RELATED FORMS

7.1 Form 3070: Fetal Bovine Serum

8.0 REFERENCES

8.1 EPA DIS/TSS-2, 25 Jan. 79 (page 2 of 3)

9.0 MOST RECENT REVISION SUMMARY

Revised 3.2.

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5/9/12

Prepared by: Lavinda Hilen Date: 3/14/11

Quality Assurance: Sherri St Clair Date: 14 Mar 2011

Management: [signature] Date: 14 Mar 2011

ECOLAB
MICROBIOLOGICAL SERVICES

Ecolab, Inc. Controlled Document Standard Operating Procedure

TITLE: **Test Substance Use-Solution Preparation for Analysis**

NUMBER: **MS088-17**

EFFECTIVE: **08/01/11**

1.0 PURPOSE

To describe the preparation and active ingredient analysis of a diluted test substance (test substance use-solution). Use-solution analysis is included with pesticide efficacy studies, chemical quality verification studies and contract lab studies to verify that the active ingredient concentration corresponds to the dilution made for the claimed active ingredient concentration in the undiluted test substance.

2.0 PROCEDURE

2.1 Typically, use-solutions are prepared as follows:

2.1.1 Use-solutions prepared according to the label are for chemical quality verification (CQV) studies

2.1.2 Use-solutions prepared at the Lower Certified Limit (LCL) are for efficacy studies

2.1.3 Use-solutions prepared at the Upper Certified Limit (UCL) are for contract lab TOX studies

2.2 Determine the concentration of active ingredient in the test substance concentrate to verify it is within claimed limits. Perform the analysis for each active ingredient in the product.

2.3 Deionized water may be used as the test substance diluent or the test substance diluent (e.g. hard/soft water or label instructed diluent) may be prepared in the same manner as used for pesticide efficacy testing.

2.4 Prepare the test substance use-solution according to label instructions or as specified in protocol using diluent as described in 2.3. This use-solution should be labeled according to M032.

Example: A 1:64 dilution is 1 part test substance, 63 parts diluent.

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TITLE: Test Substance Use-Solution Preparation for Analysis

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2.5 Analyze the test substance for active ingredient concentration using the same validated QATM that is, or will be, included in the finished good Bill of Quality (BOQ).

Note: The method used to measure active ingredient concentration in the use-solution may have limited sensitivity, accuracy and precision for quantitating the minimal levels of active ingredient found in many use-solutions. These factors may need to be considered when interpreting results. Any modifications to the QATM to adjust for this should be specified in the protocol.

2.6 Analyze the results. The active ingredient concentration in the use-solution should correspond to the dilution made for the claimed active ingredient concentration in the concentrate (e.g. EPA Upper & Lower Certified Limits) and to 40 CFR § 158.350 Certified Limits unless otherwise noted in the protocol. A scientific explanation must accompany any result which does not correspond to the dilution made for the claimed active ingredient level in the concentrate.

3.0 Formulas to Determine Use-solution Amounts and Acceptance Criteria

3.1. Dilution Factor (DF) Determination

3.1.1 Dilution Factor by Volume (DF_{vol})

Example: $\text{Dilution Factor } (DF_{vol}) = \left(\frac{1\text{ oz}}{1\text{ gallon}}\right)\left(\frac{1\text{ gallon}}{128\text{ oz}}\right) = 0.0078$

3.1.2 Density/Specific Gravity (SG) Calculation

Obtain density or specific gravity values from confidential statement of formula (CSF) or suitable documentation. Convert as necessary to g/mL or unitless for SG.

Conversion Example: $\left(\frac{9.2\text{ lbs}}{\text{gallon}}\right)\left(\frac{1\text{ gallon}}{3785.412\text{ mL}}\right)\left(\frac{453.5924\text{ g}}{1\text{ lb}}\right) = 1.102\text{ g/mL}$

$$\text{Density of Product} = \frac{\text{mass (g)}}{\text{volume (mL)}};\ \text{Specific Gravity} = \frac{\text{Density of Product}}{\text{Density of Water (1.0 g/mL)}}$$

$$\text{Density of Product} = 9.2\text{ lbs/gal} \sim 1.102\text{ g/mL};\ \text{Specific Gravity} = \frac{1.102\text{ g/mL}}{1.0\text{ g/mL}} = 1.102$$

3.1.3 $DF = DF_{vol} \times SG$

$DF = 0.0078 \times 1.102 = 0.0086$

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TITLE: Test Substance Use-Solution Preparation for Analysis

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3.2. Use-solution prepared per label (e.g. 1000 g use-solution prepared at 1 oz/gallon dilution)

3.2.1 Target mass (g) of product = [Total use-solution mass (g)] × DF

Target mass (g) of product = 1000 g × 0.0086 = 8.6 g

3.2.2 Target mass (g) of diluent = [Total use-solution mass (g)] – [Target mass (g) of product]

Target mass (g) of diluent = 1000 g – 8.6 g = 991.4 g

3.2.3 Include a range of ± 0.03 g (~ 1 drop) or ± 0.3 g (~ 10 drops) to target masses when preparing use-solutions.

Note: any appropriate total use-solution mass may be used.

3.3. Use-solution prepared at CSF lower certified limit (LCL) – 1 active ingredient

3.3.1 Determine the active ingredient concentration (ppm) in the test substance use-solution when diluted (per label or protocol) using the test substance (concentrate) with active ingredient(s) at the LCL.

Example: 1 oz/gallon

$$\%\,\text{Dilution} = \left(\frac{1\,\text{oz Product}}{1\,\text{gallon}}\right)\left(\frac{1\,\text{gallons}}{128\,\text{oz}}\right)(100\%) = 0.781\%$$

$$\text{ppm active at LCL} = \left(\frac{\%\,\text{Active at LCL}}{100\%}\right)\left(\frac{\%\,\text{Dilution}}{100\%}\right)\left(\text{Specific Gravity} \times 10^6\right)$$

$$\text{Target mass (g) of product} = \frac{\text{ppm Active at LCL} \times \text{Total mass of use - solution} \times 100\%}{10^6 \times (\%\,\text{Active Ingredient Result})}$$

3.3.2 Target mass (g) of diluent = [Total use-solution mass (g)] – [Target mass (g) of product]

Note: any appropriate total use-solution mass may be used.

3.4. Use-solution prepared from CSF lower certified limit (LCL) – multiple active ingredients

- Ensure that all active ingredients are at or below the calculated lower acceptance limit.
- This can be determined by calculating all active ingredient amounts and using an amount (of product) that ensures all active ingredients present to be less than or equal to the calculated lower acceptance limit.

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3.4.1 Follow 3.3 to determine target masses (g) of product and diluent.

Note: any appropriate total use-solution mass may be used.

3.5. Use-solution prepared at CSF upper certified limit (UCL) – 1 active ingredient

3.5.1 Follow 3.3 and replace LCL values with UCL values.

Note: any appropriate total use-solution mass may be used.

3.6. Use-solution prepared at CSF upper certified limit (UCL) – multiple active ingredients

- Ensure that all active ingredients are at or above the calculated upper acceptance limit.
- This can be determined by calculating all active ingredient amounts and using an amount that ensures any active ingredient present to be greater than or equal to the calculated upper acceptance limit.

3.6.1 Follow calculations in 3.5 (replace LCL values with UCL values) to determine target masses (g) of product and diluent.

Note: any appropriate total use-solution mass may be used.

3.7. Acceptance criteria formulas and calculations for label dilution use-solutions

3.7.1 **Example:** Product diluted at 1 oz/gallon (product/diluent)

Where: CSF UCL = 18.15%; CSF LCL = 16.43%; DF = 0.0086; Nominal (N) = 17.29%

Lower Acceptance Level = CSF LCL × DF = 16.43% × 0.0086 = 0.141%
Upper Acceptance Limit = CSF UCL × DF = 18.15% × 0.0086 = 0.156%

When the analyte of interest in the use-solution at the lower/upper acceptance limit is ≤ 1.0% after dilution; acceptance criteria may be expanded to accommodate method variability or other suitable rationale. Expanded ranges are based on 40 CFR § 158.350 (Certified Limits).

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TITLE: **Test Substance Use-Solution Preparation for Analysis**

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If the nominal concentration (N) for the ingredient is	Upper/Lower Acceptance Limits after dilution may be adjusted as follows	
	Upper Limit	Lower Limit
$N \leq 1.0\%$	N + 10%	N - 10%
$1.0\% < N \leq 20.0\%$	N + 5%	N - 5%
$20.0\% < N \leq 100.0\%$	N + 3%	N - 3%

Therefore

Lower Acceptance Limit = 0.141% - 10% → [0.141% – (0.141 × 0.1)] = 0.127%
Upper Acceptance Limit = 0.156% + 10% → [0.156% + (0.156 × 0.1)] = 0.172%

Products with CSF LCL/UCL values greater than N ± 10% should follow the same range as calculated from the CSF.

Example

Lower Acceptance Limit = 0.141% - 25% → [0.141% – (0.141 × 0.25)] = 0.106%
Upper Acceptance Limit = 0.156% + 25% → [0.156% + (0.156 × 0.25)] = 0.195%

3.8. Acceptance criteria formulas and calculations for use-solutions diluted to the CSF LCL or UCL.

3.8.1 **Example:** Product diluted to 1 oz/gallon

Acceptance criteria for use-solutions diluted to the CSF LCL or UCL are greater than or equal to the Upper/Lower acceptance limits.

Acceptance Limit (Active at CSF LCL) = CSF LCL × DF = 16.43% × 0.0086 = 0.141%
Acceptance Limit (Active at CSF UCL) = CSF UCL × DF = 18.15% × 0.0086 = 0.156%

Therefore

Acceptance Criteria (Active at CSF LCL) ≤ 0.141%
Acceptance Criteria (Active at CSF UCL) ≥ 0.156%

4.0 RELATED FORMS

4.1 Form 3113: Test Substance Use-Solution Preparation for Analysis

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TITLE: Test Substance Use-Solution Preparation for Analysis

NUMBER: MS088-17

5.0 REFERENCES

5.1 M032: Labeling Requirements
5.2 40 CFR 158.350

6.0 MOST RECENT REVISION SUMMARY

Revised 3.7.1.

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Prepared by: Laurinda Holden Date: 20 JUL 2011
Quality Assurance: Sherri St. Clair Date: 21 Jul 2011
Management: Mary Bersa Date: 21 JUL 2011

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MICROGIOLOGICAL SERVICES

TITLE: **Germicidal Spray Products as Disinfectants**

NUMBER: **MS010-20**

EFFECTIVE: **08/01/11**

1.0 PURPOSE

To determine the effectiveness of sprays and pressurized spray products as spot disinfectants for contaminated surfaces.

2.0 CULTURE MEDIA

2.1 AOAC Nutrient Broth
2.2 AOAC Synthetic Broth, containing 0.1 mL 10% Dextrose solution per 10 mL broth tube
2.3 Nutrient Agar
2.4 Sabouraud Dextrose Agar
2.5 Sabouraud Dextrose Broth
2.6 Glucose Agar
2.7 Other culture media as appropriate for test system

3.0 SUBCULTURE MEDIA

3.1 Letheen Broth
3.2 Fluid Thioglycollate Medium
3.3 Sabouraud Dextrose Broth
3.4 Sabouraud Dextrose Broth with 0.07 % lecithin & 0.5% polysorbate 80 (tween 80)
3.5 Glucose Broth
3.6 CTA Medium (Cystine Tryptic Agar)
3.7 MacConkey agar
3.8 Mannitol Salt agar
3.9 *Pseudosel agar*
3.10 Other media as appropriate for test system

4.0 APPARATUS

4.1 Test tubes: 20 × 150, 25 × 150 and 32 × 200 mm glass test tubes
4.2 Petri dishes: Glass, containing two layers of Whatman No. 2 filter paper, or equivalent
4.3 Test tube racks: Any convenient style capable of holding 20 × 150, 25 × 150 or 32 × 200 mm test tubes

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4.4 Transfer loops: Platinum-rhodium with a 4 mm inside diameter, or plastic disposable transfer loops may be used
4.5 Micropipetter and sterile pipette tips to deliver 0.01 mL
4.6 Microscope cover slips (carrier): non-corrosive, 25 × 25 mm (1" × 1") glass coverslips
4.7 Microscope slides, non-corrosive, 25 × 25 mm (1" × 1")
4.8 Metal forceps: Sharp points

5.0 REAGENTS

5.1 Diluent (refer to MS008 if preparing synthetic hard water)
5.2 Blood serum or other appropriate organic load, if applicable (refer to MS002)
5.3 0.85% Saline Solution
5.4 Triton X-100

6.0 TEST SYSTEM PREPARATION (Culture Preparation)

6.1 For *Staphylococcus aureus* ATCC 6538 and *Salmonella enterica* ATCC 10708 follow steps listed below:

6.1.1 A minimum of three consecutive transfers but less than 15 total transfers in AOAC Nutrient Broth (AOAC Synthetic Broth may also be used for *Staphylococcus aureus* and *Salmonella enterica*) need to be made before using to inoculate for testing.

6.1.2 If only one transfer is missed per seven day period, it is not necessary to repeat the three consecutive transfers.

6.1.3 If two or more transfers are missed, repeat with the three consecutive transfers.

6.1.4 Transfers of *Staphylococcus aureus* and *Salmonella enterica* are made on a 24 hour schedule. The last consecutive transfer used to inoculate for the test should be a 24 ± 4 hour test system.

6.1.5 Inoculate a sufficient number of culture media broth tubes for the test. Broth tubes should contain 20 mL of appropriate media. Incubate for 48 – 54 hours at 35 ± 2°C.

6.1.6 Vortex the 48 – 54 hour culture for three to four seconds to mix. Allow culture to stand for ten minutes. Remove the upper portion (approximately 15 mL) of the culture and use for the test.

6.1.7 Add soil challenge (blood serum and/or soap residue) to the culture if required by the test protocol (refer to MS002). Vortex to mix.

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6.2 For *Pseudomonas aeruginosa* ATTC 15442

6.2.1 A minimum of three consecutive transfers but less than 15 total transfers in AOAC Nutrient Broth (AOAC Synthetic Broth may also be used) need to be made before using to inoculate for testing.

6.2.2 If only one transfer is missed per seven day period, it is not necessary to repeat the three consecutive transfers.

6.2.3 If two or more transfers are missed, repeat with the three consecutive transfers.

6.2.4 Transfers of *Pseudomonas aeruginosa* are made on a 24 hour schedule. The last consecutive transfer used to inoculate for the test should be a 24 ± 4 hour test system.

6.2.5 Inoculate a sufficient number of culture media broth tubes for the test. Broth tubes should contain 20 mL of appropriate media. Incubate for 48 – 54 hours at 35 ± 2°C.

6.2.6 After incubation do not shake the culture but decant, aspirate or use a loop to aseptically remove the pellicle from the liquid culture. Cultures in which the pellicle has been disturbed may not be used in tests.

6.2.7 Vortex the 48 – 54 hour culture for three to four seconds to mix. Allow culture to stand for ten minutes. Remove the upper portion (approximately 15 mL) of the culture and use for the test.

6.2.8 Add soil challenge (blood serum and/or soap residue) to the culture if required by the test protocol (refer to MS002). Vortex to mix.

6.3 Other test systems may be used in this procedure. Modification of culture medium, incubation time, and incubation temperature may be necessary.

6.4 Visually inspect culture and <u>do not</u> use if culture looks atypical (e.g. contains chunks or particles).

6.5 For *Trichophyton mentagrophytes* ATCC 9533 and *Aspergillus niger* ATCC 6275

Note: Do not use culture that has been kept at or above room temperature for more than ten days as the source of inoculum for culture.

6.5.1 Inoculate the center of Glucose Agar or Sabouraud Dextrose Agar petri dishes at 26 ± 2°C for 10 to 15 days.

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6.5.2 Remove the mycelial mats from the surface of agar plates. Mats may be removed by adding 2 – 5 mL physiological saline (0.85% NaCl) to each plate, scraping the mat with an appropriate tool and then transferring suspension to a sterile glass tissue grinder or sterile Erlenmeyer flask containing glass beads. Add saline to the tissue grinder or flask and macerate by grinding (tissue grinder) or shaking flask thoroughly.

6.5.3 Filter the suspension through two layers of sterile cheesecloth to remove hyphal elements.

6.5.4 Add 0.02 mL Triton X-100 per 10 mL test system suspension to facilitate spreading on the glass slide.

6.5.5 Estimate the density of the conidial suspension by performing a plate count on the suspension using serial dilutions and pour or spread plate technique.

6.5.6 Incubate the plates at 26 ± 2°C for three to five days or until there is sufficient growth to count.

6.5.7 Standardize the conidial suspension as needed by diluting stock spore suspension (or concentrating by centrifugation, then diluting) with physiological saline so that it contains approximately 5 x 10^6 conidial/mL. The suspension may be stored at 2 – 8°C for ≤ four weeks.

7.0 CARRIER PREPARATION

7.1 Either 1" × 1" microscope slides or microscope coverslips may be used for the test. One carrier type must be used for the entire test.

7.2 Discard any carriers that are damaged.

7.3 Before sterilization, carriers must be rinsed in 95% ethanol and then rinsed in deionized water. This removes any oil or films that may be on the carriers.

7.4 Dried carriers are autoclave sterilized in glass petri dishes matted with two pieces of Whatman No. 2. or equivalent, filter paper. One carrier is used per petri dish. Carriers may be sterilized in a hot air oven for two hours at 180°C or in an autoclave steam cycle for 20 minutes with a drying cycle.

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8.0 TEST SUBSTANCE PREPARATION

8.1 Prepare (disinfectant) test substance use solutions ≤ three hours prior to use, unless otherwise specified. Prepare test substance dilution in a sterile volumetric flask according to instructions (e.g. if a 1:128 dilution is required, add 1 part test

material + 127 parts diluent). If the test substance requires dilution, use ≥ 1.0 mL or ≥ 1 g of test substance to prepare the use solution.

9.0 INOCULATION OF CARRIERS

9.1 Inoculate a sterile 1" × 1" square carrier in a glass petri dish with 0.01 mL of test system suspension. There should be one carrier in each petri dish. Vortex the test system suspension periodically during inoculation of the carriers.

9.2 Spread the culture uniformly over the entire carrier.

9.3 Record relative humidity of the incubator before the first set of carriers is dried.

9.4 Cover the petri dish and dry 30 – 40 minutes at 35 ± 2°C.

10.0 OPERATING PROCEDURE

10.1 Spray inoculated carrier for specified time and distance at regular intervals (refer to 11.1 for more information). Hold each carrier for the exposure time then drain off excess test substance and aseptically transfer each carrier at regular intervals to individual 32 × 200 mm test tubes containing 20 mL of appropriate subculture medium using sterile forceps. Shake tubes thoroughly.

10.2 Both the spraying and transfer to subculture medium should be performed ± five seconds of the actual transfer. For test substance with ≤ one minute exposure time, sprays and transfers need to be completed within ± three seconds.

10.3 If broth appears cloudy after 30 minutes, make a second subculture to fresh medium (secondary subculture).

10.4 Incubate all bacterial tubes (primary and secondary subcultures) for 48 ± 4 hours at 35 ± 2°C. *Trichophyton mentagrophytes* and *Aspergillus niger* are incubated for 10 – 15 days at 26 ± 2°C.

11.0 CONTROLS

11.1 Determination of average volume/weight delivered during product application.

11.1.1 Determine the average volume applied by spraying a petri dish at least five times with the same applicator and test substance used during efficacy testing.

11.1.2 Place a petri dish on a balance and tare. Spray the petri dish for the specified time or for the required number of trigger squeezes at the appropriate distance. Record the weight.

11.1.3 Repeat this step at least four times, for a total of ≥ five weights.

11.1.4 Average the weights to determine the average volume/weight per carrier.

Note: All controls should be incubated at 35 ± 2°C for 48 ± 4 hours for bacteria and 26 ± 2°C for 10 – 15 days for fungi.

11.2 Carrier Enumeration (CFU/Carrier)

11.2.1 Perform count for each test system used in testing. A minimum of three carriers per test system should be used.

11.2.2 Place each dried inoculated carrier into 20 mL of subculture broth.

11.2.3 Vortex the carrier/subculture medium mixture vigorously for approximately 30 seconds. Alternately, the carrier/subculture medium mixture may be sonicated for five minutes.

11.2.4 Prepare serial dilutions in Phosphate Buffer Dilution Water (PBDW) and plate in duplicate 10^{-2}, 10^{-3} and 10^{-4} dilutions using standard plating procedures. Other dilutions may be plated as appropriate.

11.2.5 A minimum of 10^4 CFU/carrier is required for a valid test. The average CFU/carrier is the average CFU/mL × 20 mL/carrier.

11.3 Viability Controls

11.3.1 Positive control

11.3.1.1 Place two dried inoculated carriers into separate tubes containing 20 mL of subculture broth. Positive growth in both tubes is required for a valid test.

11.3.2 Negative control

11.3.2.1 Place one negative carrier into 20 mL of substance broth. No growth in the tube is required for a valid test.

11.4 Test Substance Diluent

11.4.1 Subculture 1 mL of the diluent into a subculture medium test tube. No growth in the test tube is required for a valid test.

11.5 Blood Serum Sterility Control, if applicable.

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11.5.1 Subculture 1 mL of Blood Serum into subculture medium tube. No growth in the tube is required for a valid test.

11.6 Sodium Stearate Sterility Control, if applicable

11.6.1 Subculture 1 mL of Sodium Stearate into subculture medium tube. No growth in the tube is required for a valid test.

11.7 Neutralization Confirmation

11.7.1 Expose uninoculated carriers to the test substance use solution as in test. 10% of the number of carriers tested during the efficacy portion should be used for each test system.

11.7.2 Transfer carriers to subculture medium after exposure period.

11.7.3 Dilute the test system culture using PBDW to achieve approximately 100 – 1000 CFU/mL. Confirm the number of cells in the suspension by plating 1.0 mL and 0.1 mL of test organism in duplicate utilizing the pour or spread plate technique.

11.7.4 Immediately following the transfers, inoculate the tubes containing the carriers with 0.1 mL of the approximately 100 - 1000 CFU/mL suspension.

11.7.5 Neutralization is confirmed (and valid) when there is growth in all of the tubes inoculated.

ALTERNATE NEUTRALIZATION METHOD

Following incubation, randomly select at least one negative tube for each ten tubes tested. Dilute a 24 - 48 hour culture of the test system using PBDW to achieve 100 – 1000 CFU/mL. For fungi, use the conidial suspension used in the test and dilute to achieve 100 – 1000 CFU/mL. Add 0.1 mL of diluted suspension or diluted conidial suspension to each tube. Confirm number of cells in the suspension by plating 1.0 mL and 0.1 mL of test organism in duplicate utilizing the pour or spread plate technique.

11.8 Test System Purity

11.8.1 Streak the test system onto Tryptic Soy Agar with 5% Sheep's Blood or other appropriate non-selective medium. After incubation, observe for purity and perform a Gram stain, if applicable.

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12.0 OBSERVATION OF SUBCULTURE TUBES & RECORDING RESULTS

12.1 Observe each tube after incubation for absence or presence of organism growth. Growth is indicated by turbidity.

12.2 Record results as number of negative tubes/number of tubes tested.

12.3 If growth is detected, follow the verification procedure as outlined in section 13.0.

12.4 The EPA performance standard for a disinfectant requires the product to kill the test organisms on 59 out of 60 carries (or ten out of ten for *T. mentagrophytes* and *A. niger*). This standard is listed in the Pesticide Assessment Guidelines Subdivision G: *Product Performance*, USEPA 11/82, Series 91.

13.0 VERIFICATION OF TEST SYSTEM IN POSITIVE SUBCULTURE TUBES

13.1 Subculture all positive tubes to appropriate medium for the test system (see below) and incubate as appropriate.

13.2 The colony morphology should demonstrate typical characteristics as described below:

- *Staphylococcus aureus* ATCC 6538
  - TSA and Mannitol Salt Agar (MSA)
  - Large yellow colonies are present with yellow zone on MSA indicates *Staphylococcus aureus*
- *Pseudomonas aeruginosa* ATCC 15442
  - TSA and Pseudosel Agar (PA)
  - Fluorescent blue-green colony with a grape-like odor on PA indicates *Pseudomonas aeruginosa*
- *Salmonella enterica* ATCC 10708
  - TSA and MacConkey Agar (MAC)
  - Clear colony indicates non-lactose fermenting gram negative bacilli on MAC, such as *Salmonella enterica*
- *Trichophyton mentagrophytes* ATCC 9533
  - SAB Agar
  - White downy growth, yellow/white reverse

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- *Aspergillus niger* ATCC 6275
  - SAB Agar
  - Black powdery growth, light reverse

Growth on a nutrient medium, plated for each positive subculture tube, allows for further identification beyond that from the differential medium. Perform a Gram stain if further identification is necessary.

13.3 If contamination is present when verifying the positive subculture tubes, the tube containing the contaminant is considered negative for growth of that specific test system. The results would then be changed to reflect this.

14.0 RELATED FORMS

14.1 Form 3069: Germicidal Spray Products as Disinfectants

15.0 REFERENCES

15.1 2009. 6.3.04 Germicidal Spray Products as Disinfectants, 961.02, Chapter 6 Disinfectants. Official Methods of Analysis of the Association of Official Analytical Chemists
15.2 DIS/TSS-1 (January 22, 1982), Office of Pesticide Programs of the U.S. Environmental Protection Agency
15.3 DIS/TSS-2 (November 17, 1981), Office of Pesticide Programs of the U.S. Environmental Protection Agency
15.4 DIS/TSS-5 (September 22, 1982), Office of Pesticide Programs of the U.S. Environmental Protection Agency
15.5 EPA Good Laboratory Practice Standards, 40 CFR Part 160
15.6 MS002: Organic/Inorganic Soil Addition for One-Step Cleaner-Disinfectant or Sanitizer Claims
15.7 MS008: Synthetic Hard Water Preparation & Standardization
15.8 MS040: Media Preparation & Storage – Media & Chemicals

16.0 MOST RECENT REVISION SUMMARY

Revised 11.7 to include diluting the conidial suspension for use in the neutralization controls.

Prepared by: Laurinda Howe Date: 20 Jul 2011
Quality Assurance: Sherri St. Clair Date: 21 Jul 2011
Management: Mary Boeder Date: 21 Jul 2011

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Regulated Study Protocol Amendment

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Amendment Number: 1200052-1A
Amendment Effective: May 22, 2012

Description of Amendment

The following is amended into the Pesticide Efficacy Test Section of the protocol:

Statement of Proposed Statistical Method

None

Scientific Basis for Amendment

The proposed statistical method was missing from the protocol.

☒ This amendment does not affect the integrity of the study.
☐ This amendment does affect the integrity of the study.

☐ This protocol amendment has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ *Study Sponsor* ☐ *Divisional Representative* — *Date* 05.22.2012

☒ *Study Director* ☐ *Study Monitor* — *Date* 22 May 2012

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Regulated Study Protocol Amendment

Study Title:	**Aqualogic Germicidal Spray Hospital Disinfection Efficacy**
Study Number:	1200052
Amendment Number:	1200052-2A
Amendment Effective:	June 13, 2012

Description of Amendment

Aqualogic batch number 052912DT is amended into the protocol.

Scientific Basis for Amendment

At the time of protocol initiation, the batch number for this batch was unknown. The protocol is amended to identify a third batch of Aqualogic for study 1200052.

☒ This amendment does not affect the integrity of the study.
☐ This amendment does affect the integrity of the study.

☐ This protocol amendment has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ *Study Sponsor* ☐ *Divisional Representative* — *Date* 7/3/12

☒ *Study Director* ☐ *Study Monitor* — *Date* 6/13/12

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Regulated Study Protocol Amendment

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Amendment Number: 1200052-3A
Amendment Effective: July 9, 2012

Description of Amendment

The protocol is amended as follows:

Verification of Test System in Positive Subculture Tubes

All positive tubes from the test will be subcultured to Tryptic Soy Agar with 5% sheep's blood and Mannitol Salt Agar (for *S. aureus*), Pseudomonas Isolation Agar (for *P. aeruginosa*) and MacConkey Agar (for *S. enterica*).

Scientific Basis for Amendment

The protocol was amended to change the non-selective growth medium used for verification of the positive subculture tubes. The media amended in is also a non-selective growth medium able to support growth of all three test systems used in the study and a wide variety of other bacteria.

☒ This amendment does not affect the integrity of the study.
☐ This amendment does affect the integrity of the study.

☐ This protocol amendment has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ *Study Sponsor* ☐ *Divisional Representative* — *Date* 7/18/2012

☒ *Study Director* ☐ *Study Monitor* — *Date* 7/9/12

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Regulated Study Protocol Amendment

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Amendment Number: 1200052-4A
Amendment Effective Date: October 18, 2012

Description of Amendment

- The Chemical Quality Verfication section of the protocol is amended to list the CSF upper certified limit as 0.1031% free available chlorine as listed in the table below.

Active Ingredient	CSF Lower Certified Limit	CSF Upper Certified Limit
Free Available Chlorine*	0.0660%	0.1031%

*The equivalent weight of NaOCL (sodium hypochlorite) to the equivalent weight of Cl_2 (Chlorine) is 37.2/35.5 = 1.05. Dividing the sodium hypochlorite concentration by the ratio of the equivalent weight of sodium hypochlorite to the equivalent weight of chlorine results in the free available chlorine concentration.

- The Test Substance Concentration section of the protocol is amended to list the CSF upper certified limit as 0.1031% available chlorine as listed in the table below.

Active Ingredient	CSF Lower Certified Limit	CSF Upper Certified Limit
Available Chlorine	0.0660%	0.1031%

Scientific Basis for Amendment

The protocol was amended to update the upper certified limit to match a revised CSF. The previous value had a rounding error in the available chlorine value for the upper limit.

☒ This amendment does not affect the integrity of the study.
☐ This amendment does affect the integrity of the study.

☐ This protocol amendment has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ *Study Sponsor* ☐ *Divisional Representative* — *Date* 10/23/2012

☒ *Study Director* ☐ *Study Monitor* — *Date* 10/18/12

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Regulated Study Protocol Deviation

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Deviation Number: 1200052-1D
Date Deviation Occurred: May 29, 2012

Description of Deviation

On May 29, 2012, the protocol was deviated from by only subculturing positive tubes from an efficacy test to the appropriate selective medium for the test system. The protocol stated to subculture to selective media and Tryptic Soy Agar.

Justification for Deviation

A positive tube from *S. aureus* ATCC 6538 was subcultured to Mannitol Salt Agar (MSA) and a positive tube from *S. enterica* ATCC 10708 was subcultured to MacConkey Agar (MAC) from an efficacy test conducted on May 24, 2012. The subcultures were performed on May 29, 2012. The results of those subcultures were no growth. In the absence of a subculture on Tryptic Soy Agar, broth cultures of each test system were confirmed to support growth on each respective selective media. MSA was confirmed to support *S. aureus* ATCC 6538 and MAC was confirmed to support *S. enterica* ATCC 10708. This indicates that the positive tubes from test date May 24, 2012 were a contaminant for each test system. The unconfirmed result was 59 negative tubes out of 60 carriers tested for both test systems. These results would have still demonstrated disinfectant efficacy even if they were found to be the test system.

☒ This deviation does not affect the integrity of the study.
☐ This deviation does affect the integrity of the study.

☐ This protocol deviation has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ Study Sponsor ☐ Divisional Representative — 8/22/12 — Date

☒ Study Director ☐ Study Monitor — 8/21/12 — Date

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Regulated Study Protocol Deviation

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Deviation Number: 1200052-2D
Date Deviation Occurred: May 24, 2012

Description of Deviation

On May 24, 2012, an inadvertent protocol deviation occurred in the calculation of the use-solution amounts of test substance concentrate and diluent. 659 ppm should have been used in the equation listed below instead of 660 ppm as was used. The amount of test substance used should have been 1052.72 ± .03g and the diluent used should have been 447.28 ± .03g diluent.

For 1500 g solution of 051512DT-1 (24May2012 test date):

$$\frac{660 \times 100 \times 1500}{.0939\% \times 10^6} = 1054.31 \text{ g Test Substance, } 445.68 \text{ g diluent}$$

Justification for Deviation

This deviation from the protocol did not impact the overall study or the results obtained with this use-solution. The use-solution was analyzed for available chlorine content on the date prepared and was found to be at 0.0659% available chlorine. This level is within the limits specified in the protocol for the use-solution (0.0594% - 0.0726%), verifying that the calculation error did not impact the study.

☒ This deviation does not affect the integrity of the study.
☐ This deviation does affect the integrity of the study.

☐ This protocol deviation has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ *Study Sponsor* ☐ *Divisional Representative* — *Date* 09/05/2012

☒ *Study Director* ☐ *Study Monitor* — *Date* 05 Sep 2012

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Regulated Study Protocol Deviation

Study Title: Aqualogic Germicidal Spray Hospital Disinfection Efficacy
Study Number: 1200052
Deviation Number: 1200052-3D
Date Deviation Occurred: July 19, 2012

Description of Deviation

On July 19, 2012, an inadvertent protocol deviation occurred in the calculation of the use-solution amounts of test substance concentrate and diluent. The amounts of test substance and diluent listed in the raw data were found to be incorrect after the test was completed. The equation used in the calculation is listed below. The amount of test substance used should have been 431.66 ± .03g and the diluent used should have been 168.34 ± .03g diluent.

$\frac{(1699)(100)(600)}{.0916\% \times 10^6}$ = 431.39 ± .03g Test Substance
168.39 Diluent (±.03g) LAH 9/5/12

Justification for Deviation

This deviation from the protocol did not impact the overall study or the results obtained with this use-solution. The use-solution was analyzed for available chlorine content on the date prepared and was found to be at 0.0650% available chlorine. This level is within the limits specified in the protocol for the use-solution (0.0594% - 0.0726%), verifying that the calculation error did not impact the study.

☒ This deviation does not affect the integrity of the study.
☐ This deviation does affect the integrity of the study.

☐ This protocol deviation has been clarified and/or changed.
Refer to protocol amendment _____ for details.
Initial & Date _____

☒ Study Sponsor ☐ Divisional Representative — 09/05/2012 Date

☒ Study Director ☐ Study Monitor — 05 Sep 2012 Date

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